Bacteriorhodopsin: Pumping Ions

Ion Transport is Net Result of Small Changes in Protein Structure

A live cell cannot be a closed system; information and material, often in the form of ions, must be able to pass through cell membranes. Bacteriorhodopsin (BR) is a membrane-embedded protein that can actively "pump" ions from one side of the membrane to the other, against an electrochemical gradient. The energy for this process comes from a photon of visible light, which sets into motion a series of structural changes within the BR molecule that favor active ion transport. Researchers from the University of California, Irvine, have obtained atomic-resolution structures of BR crystals in two different states: one at the beginning of the ion-transport cycle and another in midstroke. Analysis of the small, but significant, structural differences between the two states provides insight into the mechanisms and forces that push and pull ions through the membrane, against the electrochemical tide.

The BR molecule contains seven helices that surround a channel through which ions can move. Charged amino-acid side chains (Glu^{204}, Glu^{194}, Arg^{82}, Asp^{85}, Asp^{96}) throughout the channel interact with the ions. Bound in a cavity roughly in the middle of the channel is a photosensitive molecule called retinal. The retinal divides the channel into a hydrophobic cytoplasmic side and a hydrophilic extracellular side. In the type of BR used in this study, the ions transported across the membrane are protons, and upon absorption of a photon of light, the retinal molecule flips toward the cytoplasmic side, losing a proton to the nearby Asp^{85} side chain. This, in turn, causes a proton to be released from the extracellular side (from Glu^{204} or Glu^{194}). Subsequently, a proton is taken up from the cytoplasmic side (via Asp^{96}), and sites that have lost a proton (such as the retinal site) are reprotonated to complete the photocycle.

In this study, the researchers used a particular BR mutant (D96N) in which the uptake of a proton from the cytoplasmic side is hindered. This enabled them to freeze the action at the M_N state (just before reprotonation of the retinal) by continuously illuminating "ground-state" crystals with yellow light. The structures of both ground-state and M_N-state crystals were then determined to 1.8 and 2.0 angstroms, respectively. This excellent resolution was made possible by the high quality of the crystals and the brightness, collimation, and small spot size of Beamline 5.0.2 at the ALS's Macromolecular Crystallography Facility.

The ground-state crystal structures show a network of amino-acid side chains and water molecules that provide a path for protons moving from the retinal to the extracellular surface. In the M_N state, the disappearance of key water molecules from this network and small shifts in the locations of side chains (in response to the displacement of the retinal) raises or lowers the proton affinity of the various binding sites. Thus, protons are released and captured by various sites, with the net result being that one proton is transported across the membrane.

This process moves in only one direction because the proton affinity of the Asp^{85}, which accepts the proton released by the retinal, remains high throughout the cycle. Also, there is no comparable network of side chains and water molecules on the cytoplasmic side to provide a continuous path from the retinal to the internal membrane surface. However, the images show the displacement of two side chains near the retinal on the cytoplasmic side, opening up a possible path for reprotonation of the retinal. Also, strong disorder in the cytoplasmic ends of two of the seven main helices suggests a possible role for these helices in the later stages of the photocycle. ■

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H. Luecke, B. Schobert, H.-T. Richter, J.-P. Cartailler, and J. K. Lanyi, "Structural Changes in Bacteriorhodopsin During Ion Transport at 2 Angstrom Resolution," *Science* **286** (1999) 255; H. Luecke, B. Schobert, H.-T. Richter, J.-P. Cartailler, and J. K. Lanyi, "Structure of Bacteriorhodopsin at 1.55 Angstrom Resolution," *J. Mol. Biol.* **291** (1999) 899.

RESEARCH FUNDING: National Institutes of Health; U. S. Department of Energy. Operation of the ALS is supported by the Materials Science Division of the U. S. Department of Energy.

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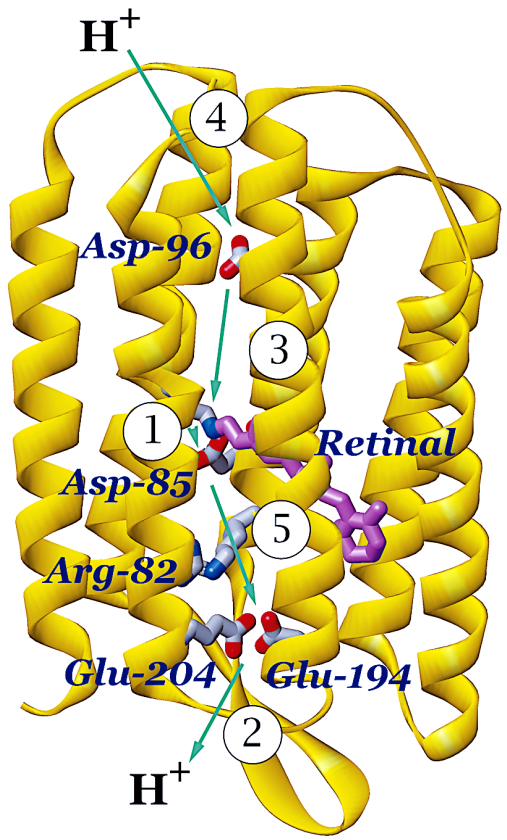

Overview of bacteriorhodopsin, including the photosensitive retinal molecule (purple) and the amino acids involved in ion transport (arrows = direction; numbers = sequence).

- **Membrane proteins**
  - *Send/receive signals, sense external stimuli, transport molecules/ions*
  - *Play large roles in disease and drug resistance*
- **Bacteriorhodopsin**
  - *Light-driven ion pump*
  - *Relatively small, simple, well-studied*
  - *The "hydrogen atom of membrane proteins"*
- **Atomic resolution (~2 Å)**
  - *Requires high-quality crystals, high-brightness x rays*
  - *Reveals small structural changes during ion pump cycle*
  - *Helps explain unidirectionality of ion pump*

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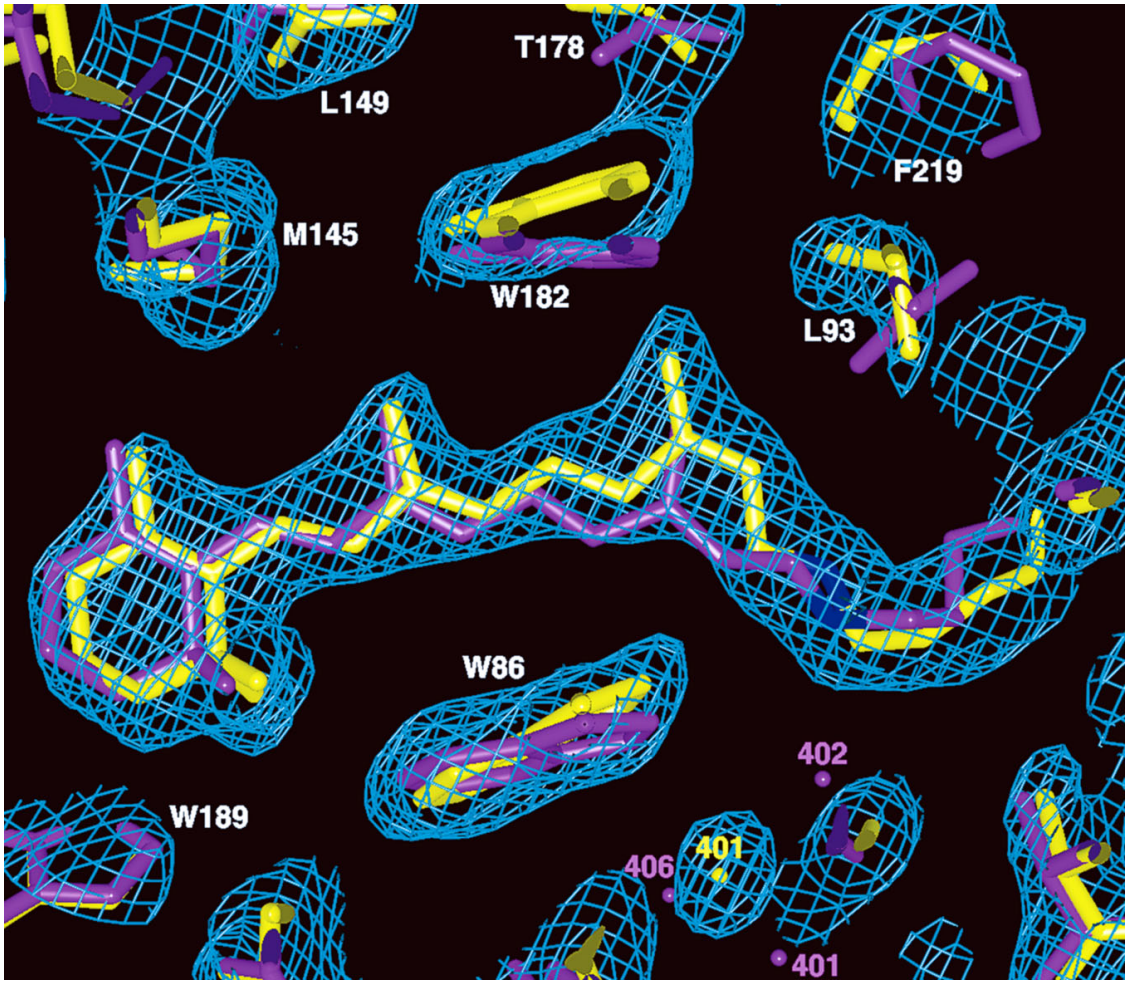

Comparison of ground-state (purple) and M_N-state (yellow) structures in the vicinity of the retinal. Small shifts in position and the disappearance of key water molecules (e.g., 402 and 406, near bottom) raise or lower the ion affinities of various ion binding sites, ensuring the unidirectionality of the ion pump. Displacements are on the order of 1 Å.

H. Luecke, B. Schobert, H.-T. Richter, J.-P. Cartailler, and J. K. Lanyi (University of California, Irvine). [*Science* **286** (1999) 255, *J. Mol. Biol.* **291** (1999) 899]